

Listing of Claims

1. (Currently Amended) A method for multigenerational plant trait analysis and associated data management comprising:

a) generating a random insertion of an insertional mutagen in the genome of a T0 plant, and collecting T1 seed from said T0 plant, wherein said insertional mutagen is capable of a loss of function and a gain of function mutation;

b) growing T1 plants from the seed collected in (a) under conditions to select transformed T1 plants, and assigning a T1 identification number to each transformed T1 plant selected;

c) analyzing a transformed T1 plant grown in (b) for dominant mutant traits and recording in an electronic database any-at least one dominant mutant traits observed in the transformed T1 plant, wherein a database record of a dominant mutant trait observed in a transformed T1 plant is linked to the T1 identification number assigned to the T1 plant analyzed; wherein the mutant trait is a morphological phenotype;

d) collecting T2 seed from the-T1 plants analyzed in (c), and assigning a T2 identification number to said seed, wherein the T2 identification number is linked to the T1 identification number assigned to the T1 plant analyzed in (c);

e) growing T2 plants from the T2 seed collected in (d); and

f) analyzing a T2 plant grown in (e) for recessive mutant traits and recording in the database any-at least one recessive mutant traits observed in the analyzed T2 plant that were-was not present in its parent T1 plant, wherein a record is generated that associates the information of the analyzed T2 plant to any information recorded about its parent T1 plant, and wherein the mutant trait is a morphological phenotype.

2. (Previously Presented) The method of Claim 1 wherein the insertional mutagen is an activation tagging vector.

3. (Original) The method of Claim 2 wherein the activation tagging vector comprises an enhancer selected from the group consisting of a multimerized CaMV 35S

enhancer, a figwort mosaic virus enhancer, a peanut chlorotic streak caulimovirus enhancer, and a mirabilis mosaic virus enhancer.

4. (Original) The method of Claim 3 wherein the enhancer is a mirabilis mosaic virus enhancer.

5. (Currently Amended) The method of Claim 1 wherein the T0 plant is selected from the group consisting of *Arabidopsis*, corn, tomato, and rice.

6. (Original) The method of Claim 1 wherein the insertion mutagen encodes a selectable marker selected from the group consisting of an antibiotic resistance gene and an herbicide resistance gene.

7. (Original) The method of Claim 1 wherein in step (b), prior to assigning T1 identification numbers to transformed plants, transformed plants are transplanted into perimeter wells of a multiwell container comprising a central well in which a barcode is provided, wherein a single perimeter well contains a single T1 plant, and wherein the T1 identification number assigned to each T1 plant in a perimeter well derives from the barcode in the corresponding central well and the relative position of the perimeter well holding said T1 plant.

8. (Original) The method of Claim 7 wherein in step (c) a hand-held electronic data entry device equipped with a barcode scanner is used by an observer to record a mutant trait observed in a T1 plant and scan the barcode in the corresponding central well such that the mutant trait observed and recorded in the hand-held electronic data entry device can be later transferred to the electronic database in association with the T1 identification number of the observed plant.

9. (Original) The method of Claim 1 wherein step (c) includes obtaining a digital image of the transformed T1 plant, entering the digital image into the database, and linking the image entry to the T1 identification number assigned to the imaged T1 plant.

10. (Cancelled)

11. (Original) The method of Claim 1 wherein the T2 plants are analyzed by performing a directed screen to identify altered resistance to an herbicide.

12. (Withdrawn) The method of Claim 1 wherein the T2 plants are analyzed by performing a directed screen to identify altered resistance to a pathogen, said pathogen selected from the group consisting of fungus, bacteria, virus, nematode, and insect.

13. (Withdrawn) The method of Claim 1 wherein the T2 plants are analyzed by performing a directed screen to identify altered stress tolerance, said stress selected from the group consisting of drought, salt, and metal.

14. (Withdrawn) The method of Claim 1 wherein the T2 plants are analyzed by performing a directed screen to identify altered level of a biochemical component, said biochemical component selected from the group consisting of vitamins, minerals, amino acids, carbohydrates, lipids, oils, nitrogenous bases, isoprenoids, phenylpropanoids, and alkaloids.

15. (Original) The method of Claim 1 wherein the mutant traits observed in (c) and (f) are recorded in the electronic database using a predefined vocabulary.

16. (Original) The method of Claim 1 wherein the T2 seed collected in (d) is distributed into a plurality of storage containers and stored under conditions that allow long-term recovery of the seeds and generation of T2 plants therefrom.

17. (Original) The method of Claim 16 wherein each of said storage containers is barcoded to relate the T2 seed contained therein with the corresponding T2 identification number used in the database.

18. (Original) The method of Claim 1 additionally comprising:
 - (g) querying the database for a specific mutant trait recorded in step (c) and/or step (f);
 - (h) obtaining T2 seed collected in (d) which is associated with the specific mutant trait queried in (g);
 - (i) performing a directed screen on the T2 seed obtained in (h) or on plants grown therefrom; and
 - (j) entering the results of the targeted screen into the database such that the targeted screen results entry is linked to the T2 identification number assigned to the T2 seed.

19. (Original) The method of Claim 18 wherein the specific mutant trait queried is a morphological trait.

20. (Previously Presented) The method of Claim 16 wherein steps (a) through (f) are repeated such that essentially every gene in the genome of the plant being analyzed is mutated by an insertional mutagen and a library of seeds that collectively represent saturation of the plant genome with insertional mutagens that generate mutant traits is generated and contained within the storage containers.

21. (Original) The method of Claim 20 wherein the T0 plant is *Arabidopsis*.

22. (Cancelled)

23. (Currently Amended) The method of Claim 1 wherein a candidate gene responsible for a specific mutant trait recorded in step (c) or step (f) is identified by additional steps comprising:

- (g) rescuing DNA flanking the insertional mutagen from a T1 or subsequent generation transformed plant;
- (h) identifying at least one candidate gene from the DNA rescued in (g); and
- (i) identifying a candidate gene identified in (h) that is over-expressed in the transformed plant.

24. (Currently Amended) The method of Claim 23 wherein the insertional mutagen comprises an enhancer element and the specific mutant trait is dominant, recorded in step (c) or step (f), wherein confirmation that the candidate gene identified in (i) causes the dominant mutant trait is achieved by additional steps comprising:

- (j) preparing a heterologous gene construct that encodes the candidate gene identified in (i) under control of a heterologous enhancer element capable of effecting mis-expression of said candidate gene;
- (k) generating a transformed test plant or explant thereof that is the same species as the T0 plant in (a) with the heterologous gene construct;
- (l) generating transformed progeny from the transformed test plant or explant thereof generated in (k) that mis-express the candidate gene; and
- (m) identifying transformed progeny generated in (l) that display the dominant mutant trait.

25. (Currently Amended) The method of Claim 24 further comprising:

- (n) transforming a test plant or explant thereof that is a different species than the T0 plant in (a) with said heterologous gene construct;
- (o) generating transformed progeny from the transformed test plant or explant thereof generated in (n) that mis-express the candidate gene; and
- (p) identifying transformed progeny generated in (o) that display the specific dominant-mutant phenotype.

26. – 33. (Cancelled)

34. (New) A method for multigenerational plant trait analysis and associated data management comprising:

- a) generating a random insertion of an insertional mutagen in the genome of a T0 plant, and collecting T1 seed from said T0 plant, wherein said insertional mutagen is capable of a loss of function and a gain of function mutation;

- b) growing T1 plants from the seed collected in (a) under conditions to select transformed T1 plants, and assigning a T1 identification number to each transformed T1 plant selected;
- c) analyzing a transformed T1 plant grown in (b) for dominant mutant traits and recording in an electronic database at least one dominant mutant trait observed in the transformed T1 plant, wherein a database record of a dominant mutant trait observed in a transformed T1 plant is linked to the T1 identification number assigned to the T1 plant analyzed, wherein the mutant trait is a morphological phenotype;
- d) collecting T2 seed from T1 plants analyzed in (c), and assigning a T2 identification number to said seed, wherein the T2 identification number is linked to the T1 identification number assigned to the T1 plant analyzed in (c);
- e) growing T2 plants from the T2 seed collected in (d); and
- f) analyzing a T2 plant grown in (e) for recessive mutant traits and recording in the database at least one recessive mutant trait observed in the analyzed T2 plant that was not present in its parent T1 plant, wherein a record is generated that associates the information of the analyzed T2 plant to any information recorded about its parent T1 plant, and wherein the mutant trait is a morphological phenotype.